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Antibody-mediated selective targeting of oral vaccines to epithelial CD13

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Introduction

Enterotoxigenic *Escherichia coli* (ETEC) are a major cause of intestinal infections in neonatal and recently weaned piglets, resulting in diarrhea, morbidity and mortality. To achieve successful immunity against intestinal pathogens, mucosal immunity must be induced (Fig.1). Oral vaccination holds much promise in this regard, but is difficult to achieve. Antigens not only have to cross the epithelial barrier, but must also circumvent the default tolerogenic immune response present in the gastro-intestinal tract.

A possible strategy to overcome these problems inherently associated with oral vaccination is by selectively delivering antigens to specific intestinal cell populations. Especially the targeting of vaccine antigens to epithelial receptors, such as CD13, looks promising in this regard (Fig.2).

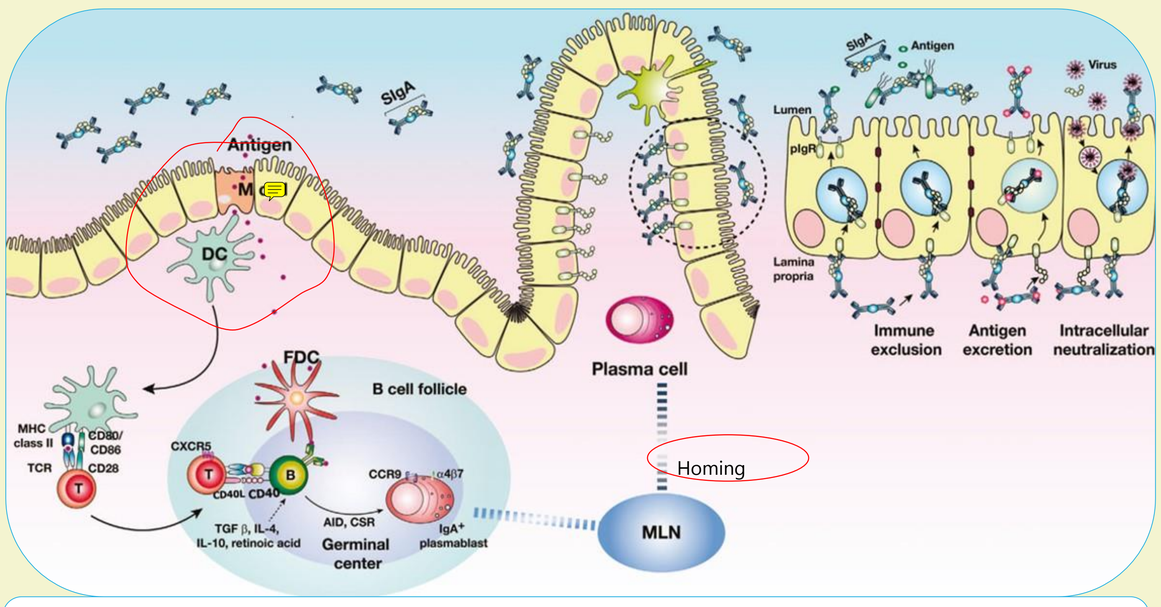

Fig.1 Intestinal mucosal immune response. Antigens are taken up by dendritic cells and presented to immune effector cells in the Peyer's patches or mesenteric lymph nodes. The mucosal immune response is characterized by the production and secretion of secretory IgA (SIgA) by plasma cells. www.b-cell-design.com

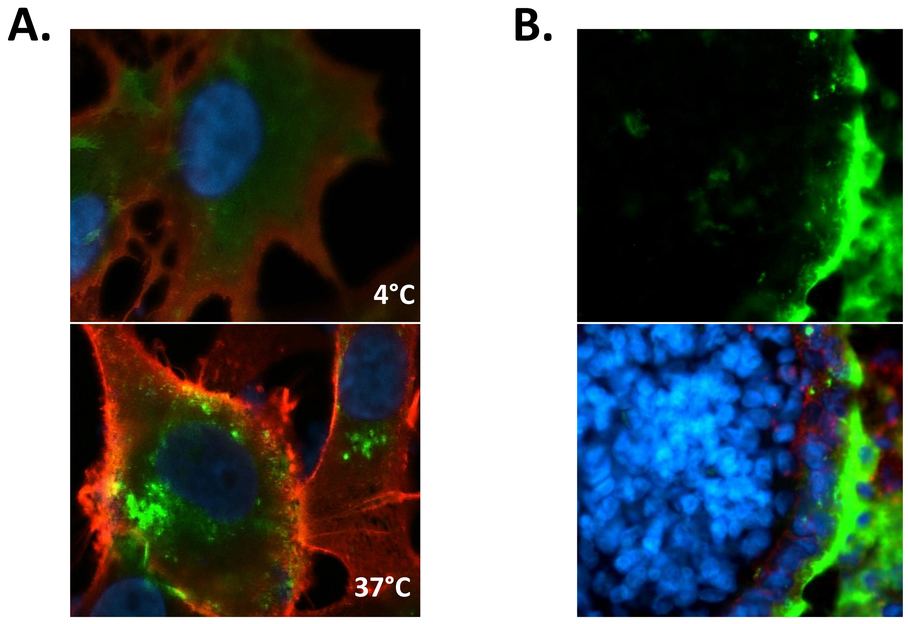

Fig.2 Endocytosis of monoclonal antibodies targeted to CD13.
(A) *In vitro* binding (Red) and endocytosis (Green) of monoclonal antibodies with a CD13-expressing cell line and **(B)** *ex vivo* binding and endocytosis of monoclonal antibodies (Green) by enterocytes (Red) in ileal explant tissue. (Blue: Hoechst)

Aim

Targeting of recombinant antibody-antigen constructs to CD13 could potentially induce a protective mucosal immune response against an antigen of interest. In this project, we aimed to develop engineered porcine IgA-Fc antibodies, linked to the FedF or TetC antigens from F18+ *E. coli* and *C. tetani* respectively, in order to induce a protective mucosal immune response towards these antigens. To enable efficient production at large scale, a novel expression system was used, combining 2A peptide cleavage and single cell sorting to assist in rapid production of our recombinant antibody constructs.

Results

A novel vector was designed that allowed the expression of GFP and antibody under a single promotor using the 2A peptide cleavage sequence (Fig.3). This design allowed the rapid selection of high producing cell lines using single cell sorting in under 2 months. Clones producing recombinant antibody at concentrations of >60 mg/L were obtained.
Several antibody-antigen constructs targeting CD13 were developed containing a porcine IgA Fc-domain, linked to the FedF or TetC antigens. These recombinant antibodies showed stable expression and similar binding and uptake characteristics, compared to the original monoclonal αCD13 antibody (Fig.4).
Next, these constructs will be assessed for their ability to induce a protective mucosal immune response against the linked antigens in an *in vivo* trial.

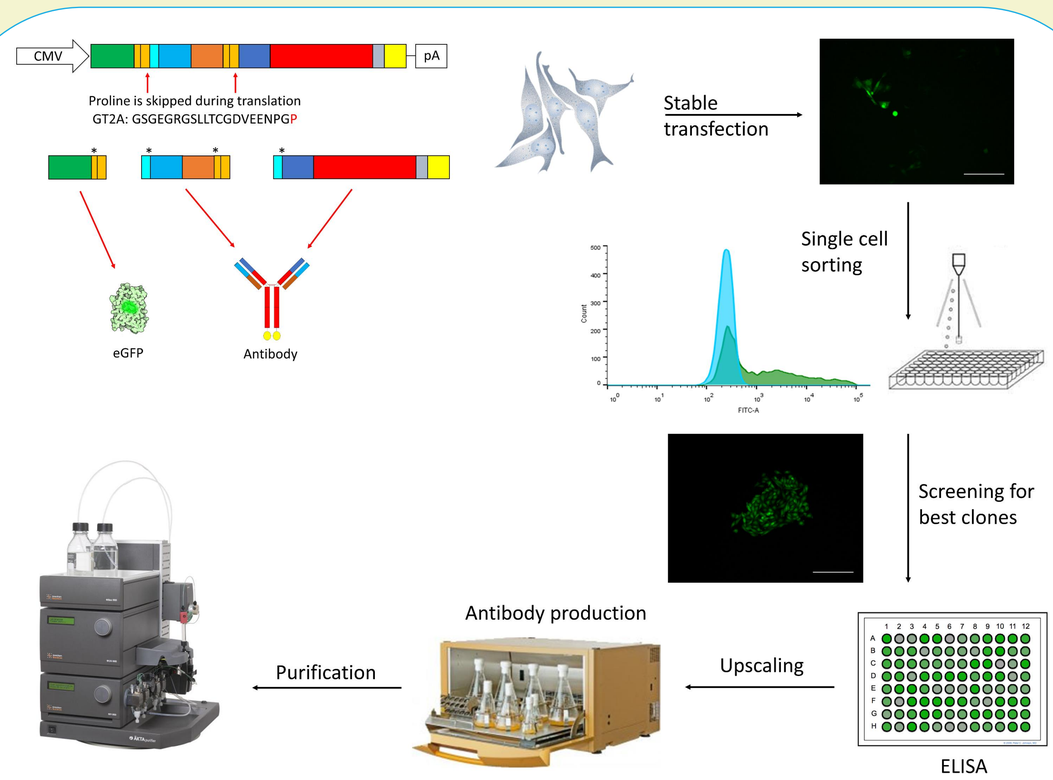

Fig.3 Recombinant antibody expression system. Starting with vector design, transfection, sorting, selection of best producing clones and antibody production + purification. *: Post-translational cleavage.
Hans Van der Weken, Eric Cox & Bert Devriendt (2019) Rapid production of a chimeric antibody-antigen fusion protein based on 2A-peptide cleavage and green fluorescent protein expression in CHO cells, mAbs, 11:3, 559-568

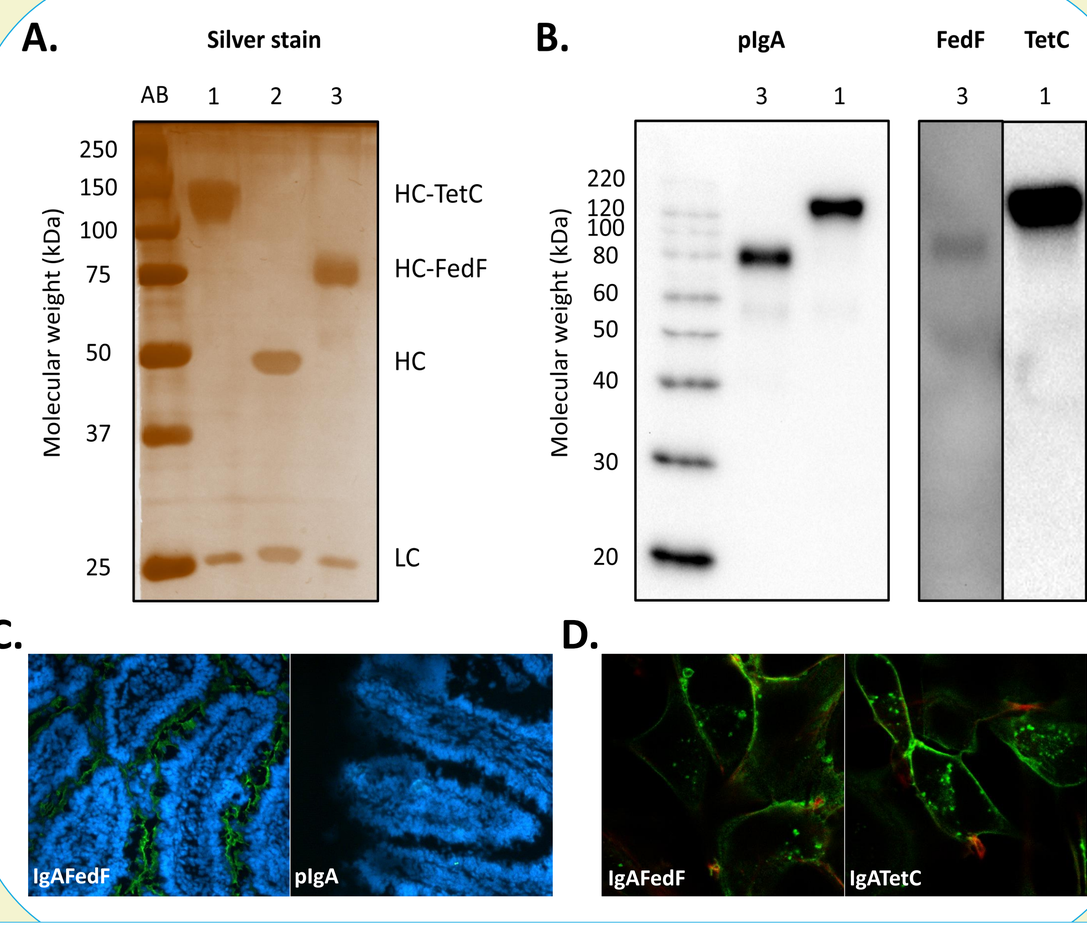

Fig.4 Recombinant antigen-antibody constructs.
(A) Silver stain and **(B)** Western blot images of recombinant antibody-antigen constructs with the porcine IgA heavy chain (HC), linked to the FedF (HC-FedF) or TetC (HC-TetC) antigens. These constructs (Green) showed stable binding and uptake on **(C)** intestinal tissue and **(D)** an APN-expressing cell line.